EXHIBIT 13

CONCENTRATIONS AND SIZE DISTRIBUTIONS OF AIRBORNE PARTICULATE MATTER AND BACTERIA IN AN EXPERIMENTAL AVIARY LAYING-HEN CHAMBER

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ABSTRACT. *High levels of airborne particulate matter (PM) and bacteria may exist in animal housing, which can be detrimental to the health of animals and workers. The sizes of these bioaerosols determine their aerial transport behaviors and depositions in the respiratory tracts of animals and humans. However, little is known regarding the size distribution of airborne PM and bacteria in livestock houses, especially in alternative animal housing systems that aim to enhance animal welfare, such as aviary hen-housing systems. The study reported here was therefore conducted to characterize the concentrations and size distributions of airborne bacteria (in count) and PM (both in count and in mass) in a pilot-scale aviary laying-hen chamber. Thirty-four laying hens were kept in the environmentally controlled aviary chamber (L × W × H = 2.2 × 2.3 × 2.4 m) for three months. The hens were given a 16L:8D photoperiod (lights on at 6:00 h and off at 22:00 h) and access to the litter floor from 12:00 h to 22:00 h daily. Airborne bacteria and PM were simultaneously sampled for 15 min at 1.5 m above the litter floor every fourth day at 5:45 h, 9:45 h, 13:45 h, 17:45 h, and 21:45 h. Concentrations of airborne bacteria at six size ranges (0.65 to 1.1 μm, 1.1 to 2.1 μm, 2.1 to 3.3 μm, 3.3 to 4.7 μm, 4.7 to 7.1 μm, and >7.1 μm) and PM concentrations (0.523 to 20.535 μm) were determined. The daily mean (±SD) concentrations of PM count, PM mass, and airborne bacteria were 1.70 (±0.66) × 10^7 particles m^{-3}, 1.12 (±0.47) mg m^{-3}, and 3.39 (±2.38) × 10^5 cfu m^{-3}, respectively. Concentrations of airborne PM and bacteria during the litter-access period (12:00 to 22:00 h) were significantly higher than those during the rest of the day when the hens were off the floor ($p < 0.05$). Median diameter and geometric standard deviation (GSD) for the PM count (0.523 to 20.535 μm) were 2.11 and 2.34 μm, respectively. Median diameter and GSD for the PM mass (0.523 to 20.535 μm) were 7.45 and 4.58 μm, respectively. PM <10 μm accounted for more than 95% of the total PM count, whereas PM >2.5 μm accounted for more than 90% of the total PM mass, in the size range of 0.523 to 20.535 μm. The majority (>95%) of the airborne bacteria were carried by particles >3.3 μm. Airborne bacteria count concentration was positively related to PM mass concentration ($p < 0.05$) with a slope of 3.84 (±2.70) × 10^5 cfu mg^{-1} PM. Results of the study are useful for improving understanding of transport behaviors of aerosols in aviary hen systems, assessing potential respiratory risks to humans and animals, and exploring mitigation techniques.*

Keywords. *Airborne bacteria, Cage-free, Particulate matter, Size distribution.*

Airborne bacteria are normally associated with particulate matter (PM) in livestock housing environments. Exposure to such airborne PM and bacteria can have negative impacts on the health of the animals and farmers (Whyte, 2002; Andersen et al., 2004; Mitchell et al., 2004). Aviary hen-housing is an alternative egg production system that features certain enrichment elements, such as litter floor, perches, and nest boxes. While hens' natural behaviors are accommodated, much higher dust and bacteria concentrations exist in aviary houses than in cage houses (Ellen et al., 2000; Protais et al., 2003; Hayes et al., 2013).

Airborne PM in livestock houses is a carrier of a large variety of microorganisms (Zhang, 2004; Lee et al., 2006; Cambra-López et al., 2010). Positive relationships between airborne PM and bacteria have been previously reported (Bakutis et al., 2004; Lai et al., 2009; Verreault et al., 2010). Airborne PM with different aerodynamic sizes may harbor bacteria. Airborne PM larger than 2 μm in diameter was found to carry high amounts of bacteria in livestock houses (Lee et al., 2006). However, little information is found on the relationship of airborne PM and bacteria con-

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centrations or the association of bacteria with particle size distributions in aviary hen housing.

Knowledge of size distributions of airborne PM and bacteria in livestock housing is conducive to understanding the transport behaviors of bioaerosols and the health risk to animals and humans, and to improving the control of indoor air quality. Several studies have investigated the size distributions of airborne PM or bacteria in broiler houses and pig houses (Heber et al., 1988; Roumeliotis and Heyst, 2007; Lai et al., 2012). However, with the continued trend toward alternative hen housing and increased use of aviary hen-housing systems, baseline information on size distributions of airborne PM and bacteria in such systems is desirable. Moreover, characterization of diurnal variations of airborne PM and bacteria concentrations is needed for improved environmental management in aviary laying-hen systems.

The objective of this study was to delineate the relationship of airborne PM and bacteria concentrations in six aerodynamic size ranges in an experimental aviary laying-hen chamber. Size distributions of the airborne PM and the associated bacteria and diurnal variations of airborne PM and bacteria concentrations in the aviary chamber were also examined.

MATERIALS AND METHODS

EXPERIMENTAL AVIARY LAYING-HEN CHAMBER

The three-month experiment was conducted in a 2.2 × 2.3 × 2.4 m environmentally controlled chamber at the Livestock Environment and Animal Physiology (LEAP) Laboratory at Iowa State University, Ames, Iowa. Thirty-four 78-week-old (onset age) CV22 laying hens were kept in the environmental chamber equipped with aviary housing components (figs. 1 and 2). A two-tier aviary setup (1.8 × 1.0 × 1.8 m) was placed in the chamber, and the floor was covered with litter (sawdust + dry manure, 1.8 × 1.8 m). The thickness of the litter (1 to 2 cm) in the chamber was based on that measured at the commercial farm where the hens were procured. The light was on at 6:00 h and off at 22:00 h (16L:8D). Hens were given access to the litter from 12:00 h to 22:00 h (10 h) of each day. The feeders, drinkers, perches, and a nest box (0.6 × 0.5 × 0.5 m) were provided in the colony cage, and the resource allowance is listed in table 1. A negative-pressure ventilation system was used that consisted of a variable-speed sidewall exhaust fan and a ceiling air inlet. A manure collection tray was placed under the cage colony, and the collected manure was scraped off and removed every four days.

EXPERIMENTAL DESIGN

Concentrations and size distributions of airborne bacteria and PM in the aviary chamber were measured at 1.5 m height above the litter floor (fig. 1). The airborne PM and bacteria were simultaneously sampled at 5:45 to 6:00 h, 9:45 to 10:00 h, 13:45 to 14:00 h, 17:45 to 18:00 h, and 21:45 to 22:00 h every fourth day (six repetitions in total).

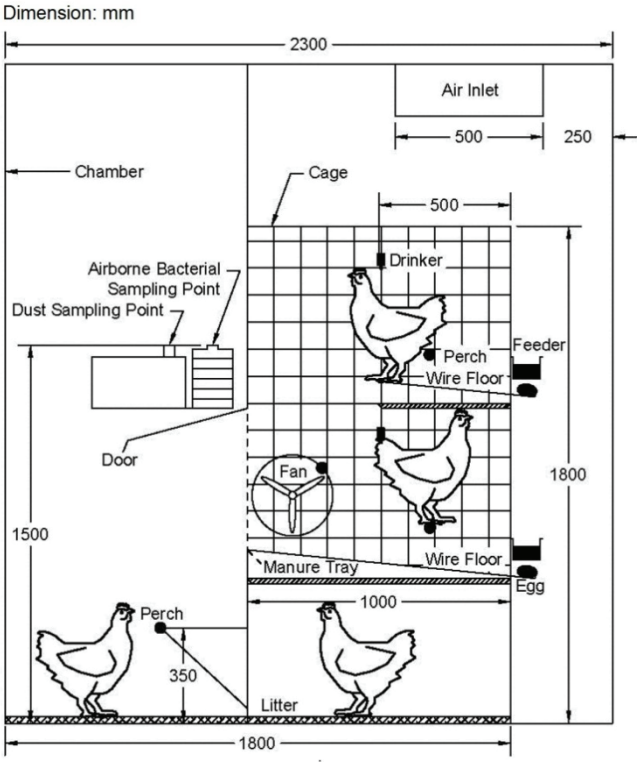

Figure 1. Cross-sectional view of the aviary laying-hen chamber.

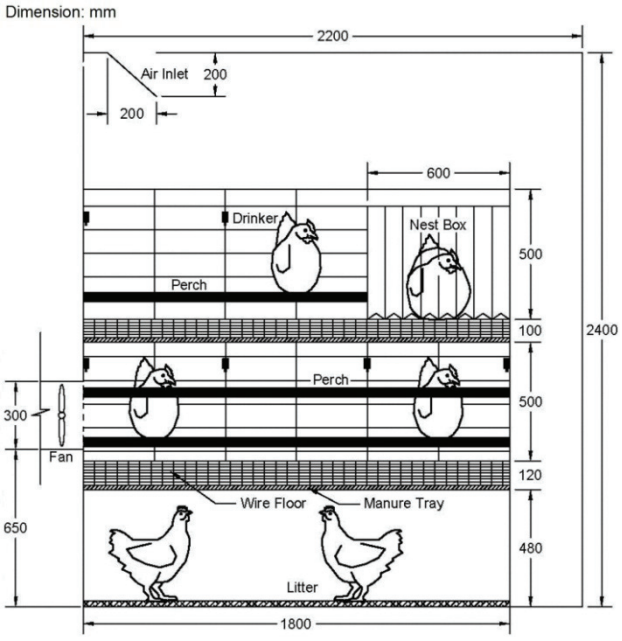

Figure 2. Longitudinal view of the aviary laying-hen chamber.

Table 1. Resource allowance in the aviary laying-hen chamber.

Wire floor	794 cm^2 $bird^{-1}$
Litter floor	953 cm^2 $bird^{-1}$
Nest space	88 cm^2 $bird^{-1}$
Perch[a]	14 cm $bird^{-1}$
Drinker	5.7 birds $drinker^{-1}$
Feed through	10 cm $bird^{-1}$
Ventilation rate	3 m^3 h^{-1} per bird
Litter moisture	15%

[a] Only the perches in the cage colony were included.

Airborne Bacteria Sampling and Analysis

A bioaerosol impactor (Six-Stage Viable Andersen Cascade Impactor, Thermo Fisher Scientific, Inc., Franklin, Mass.) was used for sampling airborne bacteria in this experiment. The impactor collects airborne microorganisms using an agar Petri dish in each of its six stages, which differentiate the collected microorganisms according to their sizes. From the first to sixth stages of the impactor, airborne microorganisms in the sizes of >7.1 µm, 4.7 to 7.1 µm, 3.3 to 4.7 µm, 2.1 to 3.3 µm, 1.1 to 2.1 µm, and 0.65 to 1.1 µm were collected. The impactor was operated at an airflow rate of 28.3 L min^{-1} and calibrated using a rotameter (RMC-123-SSV Rate-Master flowmeter, Dwyer Instruments, Michigan City, Ind.) before the experiment. Each Petri dish was filled with 27 mL of sterilized Trypticase soy-yeast extract agar (TSA) (Fisher Scientific, Pittsburgh, Pa.). The impactor was sterilized in an autoclave (121°C and 100 kPa) before the first use on a sampling day and was disinfected using alcohol cotton balls (75% alcohol) between measurements within a day.

After sampling, each Petri dish with airborne bacteria collected on the medium was immediately rinsed three times with 2 mL of sterilized 0.9% physiological saline using a sterilized spreader in a biosafety cabinet following the method described by Zhao et al. (2011b). The rinsing-off liquid received 20 µL of Tween 85 (Fisher Scientific, Pittsburgh, Pa.) to disrupt any cell-particle aggregates (Krometis et al., 2009) followed by 30 s of vortex mixing at a speed of 3000 rpm. The volume of the rinsing-off liquid sample was recorded. The liquid sample was then serially diluted (1:10) in physiological saline, and 0.5 mL of the original and the diluted samples were plated in duplicate in plastic Petri dishes with TSA agar. The plastic Petri dishes and the glass Petri dishes used in the impactor were then incubated at 37°C for 24 h. After incubation, the plastic Petri dishes with 30 to 300 visible colonies and the glass dishes were enumerated for bacterial colonies. The airborne bacteria concentration in each range was calculated using equation 1. The airborne bacteria concentrations calculated based on the duplicate counting were averaged.

$$C = \frac{\frac{N_1 V_1}{V_2} \times 10^{a} + N_2}{Qt} \quad (1)$$

where

C = airborne bacteria concentration at one of the six size ranges (cfu m^{-3})
N_1 = number of colonies in a Petri dish with 30 to 300 colonies where 10^{-a} liquid sample was cultured (cfu)
V_1 = total volume of 10^0 liquid sample (mL)
a = dilution factor of the rinsing-off liquid
V_2 = volume of 10^{-a} liquid sample cultured on TSA agar (0.5 mL)
N_2 = number of colonies in the Petri dish used in the impactor (cfu)
Q = airflow rate through the impactor with the Petri dishes (28.3 L min^{-1} = 0.0283 m^{-3} min^{-1})
t = sampling duration (15 min).

Airborne PM Measurement

The count concentration of airborne PM was determined at 5 min intervals using an Aerodynamic Particle Sizer (APS) spectrometer (model 3321, TSI, Inc., Shoreview, Minn.) that measured the particle count concentrations in 51 channels (or consecutive size ranges) over the size range from 0.523 to 20.535 µm with lower limits of 0.523, 0.562, 0.604, 0.649, 0.698, 0.750, 0.806, 0.866, 0.931, 1.000, 1.075, 1.155, 1.241, 1.334, 1.433, 1.540, 1.655, 1.778, 1.911, 2.054, 2.207, 2.371, 2.548, 2.738, 2.943, 3.162, 3.398, 3.652, 3.924, 4.217, 4.532, 4.870, 5.233, 5.623, 6.043, 6.494, 6.978, 7.499, 8.058, 8.660, 9.306, 10.00, 10.746, 11.548, 12.409, 13.335, 14.330, 15.339, 16.548, 17.783, and 19.110 µm. The PM mass concentrations in the same size ranges were also given by the APS, assuming a constant PM density of 1.0 g cm^{-3} (Lai et al., 2012). The APS was calibrated by specialists from the manufacturer before the experiment.

Data Analysis

The daily average PM count concentration, PM mass concentration, and bacteria concentration were calculated based on 30 measurements (five times a day, six days in total). For each of the five within-the-day sampling periods, airborne bacteria concentrations (in the entire size range, >0.65 µm for bacteria and 0.65 to 20 µm for PM) on the six sampling days (replications) were averaged. Statistical analysis was performed using SAS (ver. 9.2, SAS Institute, Inc., Cary, N.C.). Tukey's test was used to determine the significant differences among the means of airborne bacteria concentrations for the five sampling periods at the 5% significance level. The same methodology was used to investigate the diurnal airborne PM mass concentration variations.

The count and mass PM concentrations measured by the APS in all ranges were averaged and plotted, as is, to show the concentration distributions of PM. Since the 51 PM size ranges measured by the APS are of different spans, it is necessary to present standardized fractions in line graphs. Therefore, a spectrum of standardized PM fraction in these ranges, i.e., fraction distribution, was derived and plotted as well. The midpoint diameter was given in each size range by the APS, which was used in the distributions of standardized PM count and mass fraction. The standardized PM count and mass fraction of a size range was calculated using equations 2 and 3, respectively:

$$f_{i,count} = \frac{n_i / \Delta d_i}{N} \quad (2)$$

$$f_{i,mass} = \frac{m_i / \Delta d_i}{M} \quad (3)$$

where

$f_{i,count}$ = PM count fraction of the ith size range (µm^{-1})
n_i = particle population of the ith size range (particles m^{-3})
Δd_i = the ith size range (µm)
N = total particle population of all size ranges (particles m^{-3})
$f_{i,mass}$ = PM mass fraction of the ith size range (µm^{-1})

m_i = particle weight of the ith size range (mg m^{-3})
M = total particle weight of all size ranges (mg m^{-3}).

The count median diameter (CMD), mass median diameter (MMD), count geometric standard deviation (CGSD), and mass geometric standard deviation (MGSD) of PM over the size range of 0.523 to 20.535 µm were calculated by equations 4, 5, 6, and 7 (Zhang, 2004), respectively:

$$\mathrm{CMD} = \exp\left(\frac{\sum N_i \ln MD_i}{N}\right) \tag{4}$$

$$\mathrm{MMD} = \exp\left(\frac{\sum M_i \ln MD_i}{M}\right) \tag{5}$$

$$\mathrm{CGSD} = \exp\left(\sqrt{\frac{\sum N_i (\ln MD_i - \ln \mathrm{CMD})^2}{N-1}}\right) \tag{6}$$

$$\mathrm{MGSD} = \exp\left(\sqrt{\frac{\sum N_i (\ln MD_i - \ln \mathrm{MMD})^2}{N-1}}\right) \tag{7}$$

where

CMD = PM count median diameter (µm)
N_i = PM count number in the ith size range (particles m^{-3})
MD_i = midpoint diameter of PM in the ith size range (µm)
N = total particle population of all size ranges (particles m^{-3})
MMD = PM mass median diameter (µm)
M_i = PM mass concentration in the ith size range (mg m^{-3})
M = total PM mass of all size ranges (mg m^{-3}).
CGSD = PM count geometric standard deviation (µm)
MGSD = PM mass geometric standard deviation (µm)

To investigate the relationship between PM and airborne bacteria, concentrations of PM in the similar size range as the bacteria (i.e., 0.65 to 1.1 µm, 1.1 to 2.1 µm, 2.1 to 3.3 µm, 3.3 to 4.7 µm, 4.7 to 7.1 µm, and 7.1 to 20 µm) were calculated. PM count and mass concentrations in the range of 0.65 to 1.1 µm, 1.1 to 2.1 µm, 2.1 to 3.3 µm, 3.3 to 4.7 µm, 4.7 to 7.1 µm, and 7.1 to 20 µm were calculated using equations 8 and 9, respectively. These values were averaged based on the 30 measurements:

$$N_i = N - \frac{D_L - D_{LL}}{MD_{LH} - MD_{LL}}(N_{LH} - N_{LL}) + \frac{D_H - D_{LH}}{MD_{HH} - MD_{HL}}(N_{HH} - N_{HL}) \tag{8}$$

$$M_i = M - \frac{D_L - D_{LL}}{MD_{LH} - MD_{LL}}(M_{LH} - M_{LL}) + \frac{D_H - D_{LH}}{MD_{HH} - MD_{HL}}(M_{HH} - M_{HL}) \tag{9}$$

where

N_i and M_i = PM count concentration and PM mass concentration in the ith range of 0.65 to 1.1 µm, 1.1 to 2.1 µm, 2.1 to 3.3 µm, 3.3 to 4.7 µm, 4.7 to 7.1 µm, and 7.1 to 20 µm, respectively (particles m^{-3}, mg m^{-3})
N and M = PM count concentration and PM mass concentration in the range of 0.649 to 1.075 µm, 1.075 to 2.054 µm, 2.054 to 3.162 µm, 3.162 to 4.532 µm, 4.532 to 7.499 µm, and 7.499 to 19.110 µm, respectively (particles m^{-3}, mg m^{-3})
D_L = lower diameter boundary of the ith size range, i.e., 0.65 µm for 0.65 to 1.1 µm (µm)
D_{LL} = diameter lower than the lower diameter boundary of the ith size range, i.e., 0.649 µm, 1.075 µm, 2.054 µm, 3.162 µm, 4.532 µm, and 7.499 µm, respectively (µm)
MD_{LH}, N_{LH}, and M_{LH} = PM midpoint diameter, PM count concentration, and PM mass concentration in the range of 0.649 to 0.698 µm, 1.075 to 1.155 µm, 2.054 to 2.207 µm, 3.162 to 3.398 µm, 4.532 to 4.870 µm, and 7.499 to 8.058 µm, respectively (µm, particles m^{-3}, and mg m^{-3})
MD_{LL}, N_{LL}, and M_{LL} = PM midpoint diameter, PM count concentration, and PM mass concentration in the range of 0.604 to 0.649 µm, 1.000 to 1.075 µm, 1.911 to 2.054 µm, 2.943 to 3.162 µm, 4.217 to 4.532 µm, and 6.978 to 7.499 µm, respectively (µm, particles m^{-3}, and mg m^{-3})
D_H = upper diameter boundary of the ith size range, i.e., 1.1 µm for 0.65 to 1.1 µm
D_{LH} = diameter lower than the upper diameter boundary of the ith size range, i.e., 1.075 µm, 2.054 µm, 3.162 µm, 4.532 µm, 7.499 µm, and 19.110 µm, respectively (µm)
MD_{HH}, N_{HH}, and M_{HH} = PM midpoint diameter, PM count concentration, and PM mass concentration in the range of 1.075 to 1.155 µm, 2.054 to 2.207 µm, 3.162 to 3.398 µm, 4.532 to 4.870 µm, 7.499 to 8.058 µm, and 19.110 to 20.535 µm, respectively (µm, particles m^{-3}, and mg m^{-3})
MD_{HL}, N_{HL}, and M_{HL} = PM midpoint diameter, PM count concentration, and PM mass concentration in the range of 1.000 to 1.075 µm, 1.911 to 2.054 µm, 2.943 to 3.162 µm, 4.217 to 4.532 µm, 6.978 to 7.499 µm, and 17.783 to 19.110 µm, respectively (µm, particles m^{-3}, and mg m^{-3}).

The percent of PM count and PM mass in each size range to those in the entire size range (0.65 to 20 µm) was calculated using equations 10 and 11:

$$P_{i,count} = \frac{N_i}{N} \tag{10}$$

$$P_{i,mass} = \frac{M_i}{M} \tag{11}$$

where

$P_{i,count}$ = percent of ith size range in the entire size range in count (%)

N_i = number of PM count in the ith size range (particles m^{-3})
N = sum of PM count in the entire size range (particles m^{-3})
$P_{i,mass}$ = percent of ith size range in the entire size range in mass (%)
M_i = PM mass in the ith size range (mg m^{-3})
M = sum of PM mass in the entire size range (mg m^{-3}).

For each size range of 0.65 to 1.1 µm, 1.1 to 2.1 µm, 2.1 to 3.3 µm, 3.3 to 4.7 µm, 4.7 to 7.1 µm, and >7.1 µm (7.1 to 20 µm for airborne PM), airborne PM and bacteria concentrations based on the 30 measurements were recorded, respectively. To investigate the relationship between airborne PM and bacteria concentration, bivariate correlation analysis was performed using SAS (ver. 9.2, SAS Institute, Inc., Cary, N.C.) at the 5% significance level. Linear regression equations were developed with Microsoft Excel (Microsoft Corp., Redmond, Wash.).

The bacteria concentrations related to airborne PM mass in each size range were calculated using equation 12. For each size range, the bacteria concentrations related to airborne PM mass based on the 30 measurements were averaged. Statistical analysis was performed using SAS (ver. 9.2, SAS Institute, Inc., Cary, N.C.). Tukey's test was used to determine the significant differences among the means of airborne bacteria concentrations related to airborne PM mass in the six size ranges at the 5% significance level:

$$C_i = \frac{C_b}{C_p} \quad (12)$$

where
C_i = bacteria concentration related to airborne PM mass in the ith size range (cfu mg^{-1})
C_b = airborne bacteria concentration in the ith size range (cfu m^{-3})
C_p = airborne PM concentration in the ith size range (mg m^{-3}).

Results and Discussion

Concentrations of Airborne PM and Bacteria

During the experiment, ventilation rate was maintained at about 3.0 m^3 h^{-1} per bird. Air temperature varied from 19.0°C to 26.3°C (averaging 21.6°C), and relative humidity varied from 22% to 68% (averaging 37%) in the environmental chamber. The daily average PM count concentration, PM mass concentration, and bacteria concentration were 1.70 (±0.66) × 10^7 particles m^{-3}, 1.12 (±0.47) mg m^{-3}, and 3.39 (±2.38) × 10^5 cfu m^{-3}, respectively. Airborne PM and bacteria concentrations can be affected by many factors during the experiment, such as air temperature, relative humidity, and bird activity. Airborne PM and bacteria concentrations vary during a day or on different sampling days. The large variation of relative humidity may result in considerable variation of PM and bacteria concentration in the chamber, which should be considered in future studies.

As shown in figure 3, the airborne PM and bacteria concentrations showed similar diurnal patterns during the sampling periods. The highest airborne PM and bacteria concentrations occurred at 13:45 to 14:00 h, followed by 17:45 to 18:00 h, 21:45 to 22:00 h, 9:45 to 10:00 h, and 5:45 to 6:00 h. The airborne PM and bacteria concentrations during the litter-access period (12:00 to 22:00 h) were significantly higher than those during the off-litter period ($p < 0.05$). Some researchers reported that dust and bacteria concentrations in the day were higher than at night in laying-hen perchery systems (Takai et al., 1998; Seedorf et al., 1998). Bird activity is a major cause for airborne PM concentration changes in poultry houses (Heber et al., 2006; Mitchell et al., 2004; Zheng et al., 2012). Litter is a major source of airborne PM and bacteria (Vucemilo et al., 2007, Zhao et al., 2013). Barker et al. (2010) reported aerobic bacteria concentrations in poultry litter of more than 7.0 $\log_{10}$ cfu (g litter)$^{-1}$. These high concentrations of airborne bacteria and PM during the litter-access period are attributable to the high bird activities on litter.

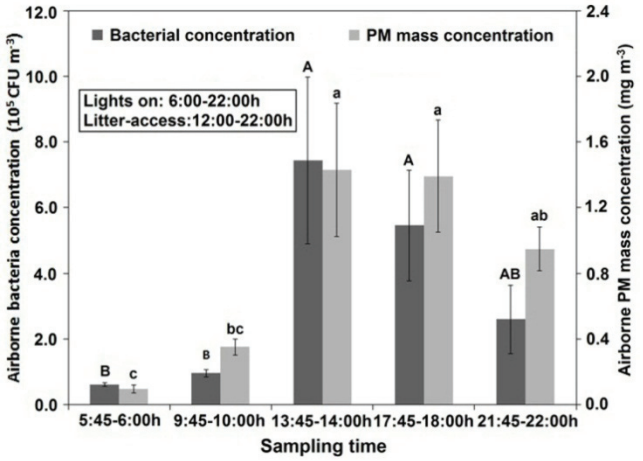

Figure 3. Diurnal variations of airborne PM and bacteria concentrations in the experimental aviary hen chamber. Vertical bars represent SE. Vertical bars labeled with different letters in the same series indicate significant difference ($p < 0.05$).

Size Distributions of Airborne PM and Bacteria

Size distributions of airborne PM in count and in mass (0.523 to 20.535 µm) are shown in figures 4 and 5, respectively. Over the entire size range of 0.523 to 20.535 µm, the PM count had a median diameter of 2.11 µm and a GSD of 2.34 µm, while the PM mass had a median diameter of 7.45 µm and a GSD of 4.58 µm. It is apparent that the two distributions differ considerably. The standardized count fraction was high in the size range of 0.523 to 1.0 µm and then decreased with increasing diameter. Specifically, PM_1, $PM_{1\text{-}2.5}$, $PM_{2.5\text{-}10}$, and $PM_{10\text{-}20}$ accounted for 26.6%, 28.5%, 42.4%, and 2.5% in count, respectively, over the range of 0.523 to 20.535 µm, with $PM_{2.5}$ dominating the distribution (55.1%). Lai et al. (2012) reported that for the particle count range of 0.25 to 32 µm in animal houses (including broiler, layer, turkey, pig, dairy, and mink houses), PM_1 accounted for an average of 87% of the total count. The high $PM_{1\text{-}10}$ proportion in count in the aviary hen chamber resulted from the presence and use of litter by the hens for scratching and dust-bathing. The standardized mass frac-

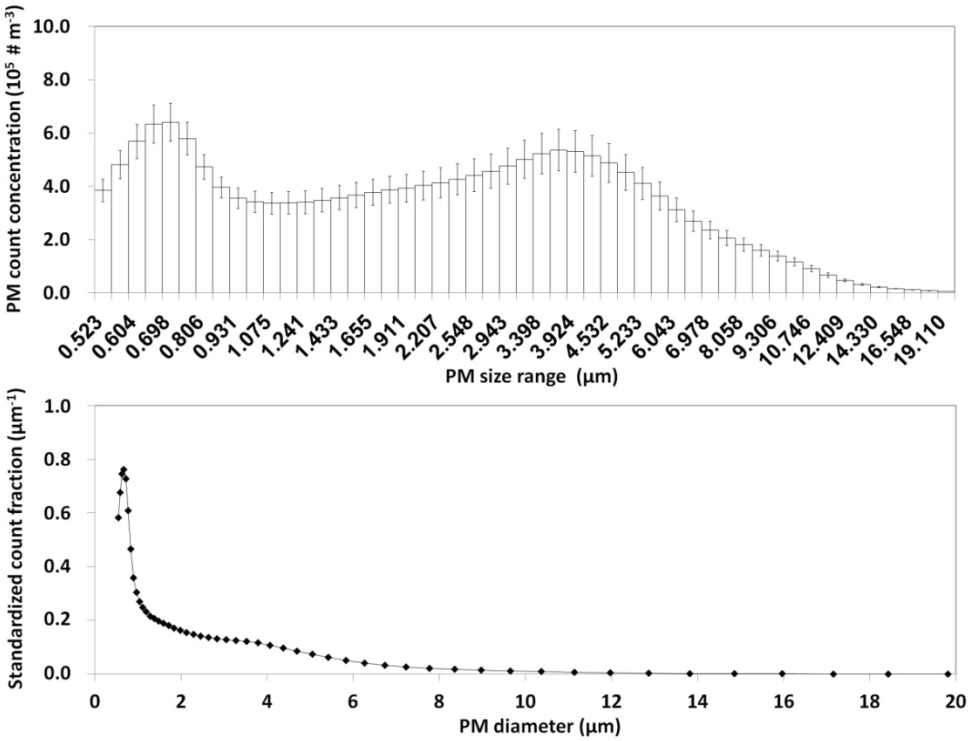

Figure 4. Airborne PM count concentrations in the 51 size ranges and standardized count fraction in the range of 0 to 20 µm. Vertical bars represent standard errors.

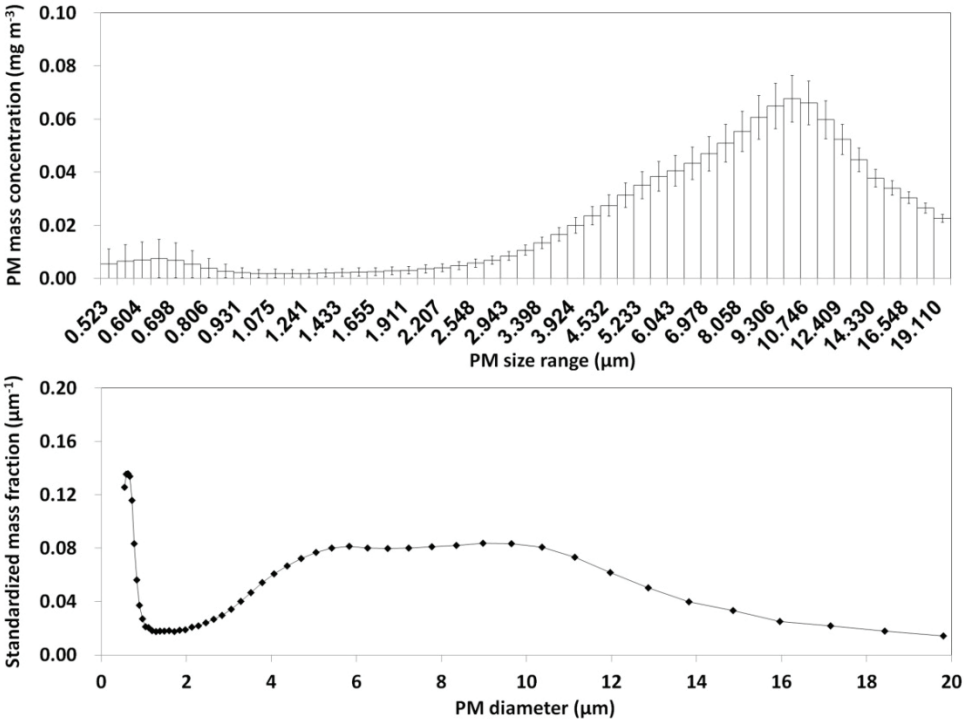

Figure 5. Airborne PM mass concentrations in the 51 size ranges and standardized count fraction in the range of 0 to 20 µm. Vertical bars represent standard errors.

tion was high in the size ranges of 0.523 to 1.0 µm and 4 to 12 µm. Specifically, $PM_{2.5}$ and PM_{10} accounted for 7% and 61%, respectively, in mass over the range of 0.523 to 20.535 µm. Wang-Li et al. (2013) reported that $PM_{2.5}$ and PM_{10} accounted for 5% to 9% and 23% to 39% in mass, respectively, over the range of 0.4 to 2000 µm. The high proportion of PM_{10} in mass in the aviary hen chamber could have resulted from the low upper limits of the APS (20 µm) and/or the activities of the hens on the litter. Size distributions of airborne PM and bacteria in the aviary lay-

Table 2. Size distributions of airborne bacteria concentration, airborne PM mass, and PM count concentration in the six size ranges.

Size Range (μm)	Bacteria Concentration (10^4 cfu m^{-3})		PM Mass Concentration (10^{-2} mg m^{-3})		PM Count Concentration (10^6 particles m^{-3})	
	Mean ±SE	Percent	Mean ±SE	Percent	Mean ±SE	Percent
0.65 to 1.1	0.018 ±0.004	0.1	0.092 ±0.019	0.1	3.16 ±0.71	24.7
1.1 to 2.1	0.176 ±0.034	0.5	0.463 ±0.068	0.5	2.12 ±0.31	16.6
2.1 to 3.3	0.70 ±0.15	2.0	1.89 ±0.31	2.0	1.81 ±0.29	14.2
3.3 to 4.7	4.14 ±1.31	11.9	7.72 ±1.91	8.3	2.33 ±0.57	18.2
4.7 to 7.1	8.36 ±2.52	24.1	25.70 ±6.61	27.6	2.36 ±0.61	18.4
>7.1[a]	21.31 ±3.87	61.4	57.18 ±9.82	61.5	1.02 ±0.19	8.0

[a] The size range of airborne PM is 7.1 to 20 μm.

ing-hen chamber were investigated under the upper limit of 20.535 μm as a result of the APS's limitation. Examining size distributions of PM and bacteria for the whole size range in aviary houses is desired in future studies.

Particulate matter count concentrations, PM mass concentrations, and airborne bacteria concentrations in each subrange and the corresponding percentage of each subrange relative to the entire size range are listed in table 2. The PM count distributions in the subranges were largely uniform, with the exceptions of the lower (0.65 to 1.1 μm) and upper (7.1 to 20 μm) subranges accounting for a larger and smaller proportion (24.7%, 8.0%), respectively. In contrast, the PM mass distributions depended on the particle size, with the smaller diameter range having the least share (0.1%) and the largest diameter range having the greatest share (61.5%) in the six subranges. As shown in table 2, size distribution of airborne bacteria essentially mirrored PM mass distribution. This result was consistent with the study of pig housing by Zhao et al. (2011a), who found that airborne bacteria were predominantly associated with particles >3.3 μm. It is speculated that airborne PM (carriers of airborne bacteria) with larger aerodynamic size and higher mass contains more bacteria. A particle with larger aerodynamic size has a larger surface area and greater mass, which might provide more space, water, and nutrition for bacteria.

Relationship of Airborne PM and Bacteria

The airborne bacteria concentration (cfu m^{-3}) and PM mass concentration (mg m^{-3}) followed linear relationships ($p < 0.05$) for all the ranges. No significant differences in such relationships were detected among the subranges; hence, the data were pooled to plot the linear relationship ($r^2 = 0.86$) for the entire range (fig. 6). Airborne PM is considered the carrier of airborne bacteria, and airborne PM in livestock buildings contains a large variety of bacteria (Zhang and Chen, 2006; Lee et al., 2006). The more PM suspends in the air, the more bacteria exist in the air. The linear relationship between airborne bacteria concentration and PM mass concentration varied and could possibly be affected by several factors, such as air temperature, relative humidity, and source of the PM. Relative humidity is relevant to the moisture content of PM. Source of the PM is related to its size and chemical composition (Cambra-

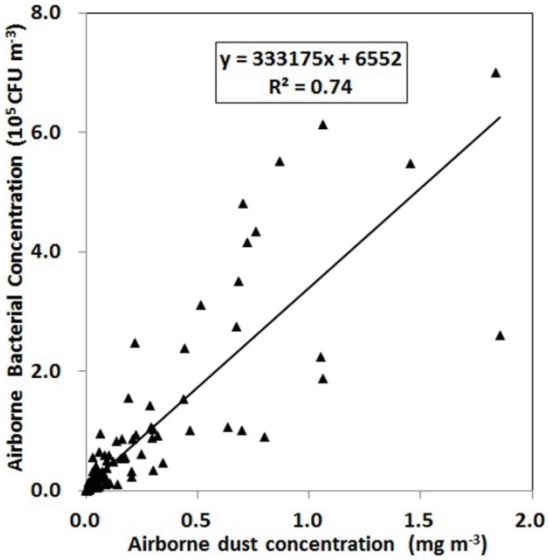

Figure 6. Relationship between airborne bacteria concentration and airborne PM concentration in the range of 0.65 to 20 μm.

López et al., 2011). They all are the living conditions of bacteria, and future study is expected to investigate how they can affect the relationship between airborne bacteria and PM. PM >20 μm was not measured in this experiment due to the instrument limit, realizing that PM >20 μm can be hardly suspended in the air. In this study, PM of 7.1 to 20 μm was taken as PM >7.1 μm when assessing the relationship between airborne PM and bacteria concentrations. Airborne bacteria concentrations related to airborne PM mass in different size ranges are shown in table 3. The specific bacteria concentration related to airborne PM mass in the range of 0.65 to 20 μm was 3.84 (±2.70) × 10^5 cfu mg^{-1} PM. No significant differences were detected among the subranges ($p = 0.21$).

Conclusions

The following conclusions were drawn from the current study that delineates distributions and relationships of airborne PM and bacteria in an experimental aviary hen chamber.

Table 3. Airborne bacteria concentrations related to airborne PM mass in different size ranges (mean ±SD, *n* = 30).[a]

	Size Range (μm)						
	0.65 to 1.1	1.1 to 2.1	2.1 to 3.3	3.3 to 4.7	4.7 to 7.1	>7.1	>0.65
Bacteria concentration (105 cfu mg^{-1})	3.19 ±2.99	3.50 ±2.07	3.27 ±2.34	4.54 ±2.98	4.00 ±3.22	4.45 ±2.39	3.84 ±2.70

[a] No significant differences were detected among the sizes ($p = 0.21$).

- Airborne PM and bacteria concentrations during the period of litter access by the hens (12:00 to 22:00 h) were significantly higher than those during the off-litter period ($p < 0.05$).
- In the size range of 0.65 to 20 µm, median diameter and geometric standard deviation (GSD) for the PM count were 2.11 and 2.34 µm, respectively, and median diameter and GSD for the PM mass were 7.45 and 4.58 µm, respectively. PM <10 µm accounted for more than 95% of the total PM count, whereas PM >2.5 µm accounted for more than 90% of the total PM mass.
- Airborne bacteria count in the aviary laying-hen chamber was positively related to PM mass concentration ($p < 0.05$) with a slope of 3.84 ($\pm$2.70) $\times 10^5$ cfu mg^{-1} PM for the aerodynamic size range of 0.65 to 20 µm.
- The majority (>95%) of the airborne bacteria were carried by particles >3.3 µm.

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